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The cytochrome P450IIC (previously designated P-450PBc) subfamily contains several closely related members. Prior to this grant, cDNA's for three of these members had been isolated and sequenced and a portion of one of the genes, cytochrome P450IIC2, had been characterized. During this granting period, a fourth cDNA for cytochrome P450IIC4 was identified and characterized, genomic fragments, including the 5' flanking regions, were characterized for three other genes, the induction of cytochrome P450IIC4 by phenobarbital was demonstrated, induction by phenobarbital of cytochrome P450IIC1/2/4 was shown to be largely accounted for by an increase in transcription rates, the probable correspondence of P450IIC2 to kidney cytochrome P450K was established, and the number of closely related genes in the rabbit P450 subfamily was estimated at about 10 on the basis of the size and number of bands in a Southern analysis. Attempts to obtain expression of hybrid genes containing P450IIC 5' flanking regions and a reporter gene in several cell lines were unsuccessful.

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FINAL TECHNICAL REPORT

RESEARCH GRANT AFOSR 84-317

September 1, 1984 to August 30, 1988

SUMMARY

The cytochrome P450IIC (previously designated P-450PBc) subfamily contains several closely related members. Prior to this grant, cDNA's for three of these members had been isolated and sequenced and a portion of one of the genes, cytochrome P450IIC2, had been characterized. During this granting period, a fourth cDNA for cytochrome P450IIC4 was identified and characterized, genomic fragments, including the 5' flanking regions, were characterized for three other genes, the induction of cytochrome P450IIC4 by phenobarbital was demonstrated, induction by phenobarbital of cytochrome P450IIC1/2/4 was shown to be largely accounted for by an increase in transcription rates, the probable correspondence of P450IIC2 to kidney cytochrome P450K was established, and the number of closely related genes in the rabbit P450 subfamily was estimated at about 10 on the basis of the size and number of bands in a Southern analysis. Attempts to obtain expression of hybrid genes containing P450IIC 5' flanking regions and a reporter gene in several cell lines were unsuccessful.

A. RESEARCH OBJECTIVES

The overall goal and the specific aims of this project remained basically the same as originally proposed. The overall goal is to understand the regulated biosynthesis of rabbit liver cytochrome P450. To achieve this goal, we are studying the structure of the genes for these enzymes, the mechanisms by which phenobarbital induces the activity of the enzymes and the nature of the mRNA's. One of the original goals to "isolate cloned cDNAs of other rabbit liver P450s that cannot be detected by hybridization to previously cloned cDNAs (years 3+)" was eliminated because the P450IIC2 subfamily genes are sufficient for our studies on phenobarbital induction. Each of the specific aims is listed below:

1. Examine whether the P-450IIC cDNAs code for actual P-450's.

2. Isolate other cloned cDNAs in the (P450IIC) subfamily or other P450 subfamilies that can be detected by hybridization with P450IIC cDNA probes.

3. Estimate the number of genes related to P450IIC genes by Southern hybridization.

4. Isolate and characterize cloned DNA fragments containing the P450IIC genes.

5. Evaluate the evolutionary relationships and possible functional significance of structure by comparative analysis of amino acid and nucleotide sequences of the P450IIC proteins with each other and other P450's.

6. Analyze the regulation of the transcription of the P450IIC genes introduced into mammalian cells.

7. Analyze the number and size of nuclear and cytoplasmic RNA's that hybridize to P450IIC cDNA probes.

8. Analyze the kinetics of the induction of P450IIC mRNAs.

9. Analyze the tissue distribution and tissue specificity of the induction of P450IIC mRNAs.

10. Determine the role of synthesis and degradation in the induction of P450IIC mRNA.

B. PROGRESS REPORT

The progress made for each of the specific aims listed on the previous page is summarized below:

1. Examine whether the P-450PBc cDNAs code for actual P-450's. Several developments in the P450 field, as well as our own research, have confirmed that the proteins encoded by the P450IIC cDNAs code for authentic P450s.

P450IIC2 was shown to be analogous to a P450 characterized in the kidney by Eric Johnson and his colleagues. Like P450IIC2, P450K was present in liver and kidney and was moderately induced by phenobarbital. In addition an antisera to P450IIC5 which reacted with P450K also crossreacted with a fusion protein of the bacterial P-450, P-450CAM, and P-450PBc2 which we had produced in bacteria. To further compare these two proteins, a full length P-450 cDNA was constructed by fusing the region coding for the N-terminal amino acids from the genomic clone to the near full length cDNA clone for P-450IIC2. This full length clone was inserted into pTZ18R behind a T7 RNA polymerase promoter. This construction allowed large amounts of mRNA for P-450IIC2 to be produced in vitro. This RNA was then sent to Dr. Johnson's laboratory and translated into protein and the properties of the protein was compared with those of P-450K. The in vitro translation product could be immunoprecipitated with two antisera, 2F5 and 3C3, which also recognize P-450K. Neither protein reacted with a third more specific antisera, 1F11, which had also been developed against P450IIC5. In addition the mobilities of immunoprecipitated P-450K and the P-450IIC2 translation products were identical when analyzed by electrophoresis on SDS-acrylamide gels. Finally Dr. Johnson's lab determined the N-terminal sequence of immunoprecipitated P-450K which was identical to that of P-450IIC2. These data establish with reasonable certainty that P-450IIC2 cDNA codes for P-450K. This work has been published (Ref. 3).

The partial protein sequence derived from the cloned P450IIC3 cDNA was shown to be nearly equivalent (>98% identical) to rabbit P450(3b) by comparison of the sequence derived from the cDNA with that determined from the P450(3b) protein by Ozols and Johnson. The characterization of the genomic clone for P450IIC3 allowed us to compare the remainder of the derived protein sequence with that determined for the protein, P450(3b), which corresponds to P450IIC3. The table below summarizes the differences between the sequences derived from the gene, cDNA and protein. Several differences are noted that may result from polymorphisms or from errors in sequencing. The close correspondence between

TABLE 1

	Amino Acid Position								
	49	82	84	89	223	256	338	342	429
Protein	Asp	Gly	Ile	Tyr	Gly	Ser	Ser	Ser	Thr
cDNA	Asn	Thr	Lys	Asp	gap	Ser	Met	Thr	Ala
Gene	Asn	Thr	Lys	Asp	gap	Leu	Met	Thr	Ala

the gene and cDNA sequence suggest that several misassignments of the protein sequence were made. Part of this information has been published (ref. 5) and the remainder will be published with the total gene structure.

2. Isolate other cloned cDNAs in the P450IIC subfamily or other P450 subfamilies that can be detected by hybridization with P450IIC cDNA probes. Prior to the initiation of this grant, three members of the P450IIC subfamily had been characterized. A partial cDNA clone for a fourth member (P450IIC4) of the P450IIC subfamily was isolated and characterized. The DNA sequence of the cDNA insert was determined and about 400 bp from the C-terminal coding region and 400 bp from the 3′ untranslated region were present. P450IIC4 was about 80% homologous to P450IIC1 and 2 and about 65% homologous to P450IIC3. It was very closely related to P450IIC5 with about 98% homology. Differences in the 3′ noncoding region suggested that these two closely related sequences represent different genes rather than different alleles of the same gene. This work has been published (ref. 4).

3. Estimate the number of genes related to P450IIC genes by Southern hybridization. In order to estimate the number of members of the P450IIC subfamily, genomic DNA was analyzed by Southern blotting using either full-length P-450IIC cDNA or the cDNA coding for the 3′-untranslated region as probes. When full-length inserts of P-450s IIC1-4 were used as probes several bands were observed. With P-450IIC2 full-length cDNA as the hybridization probe, Hind III fragments of 5.4-kb, 4.0-kb and 2.7-kb were observed as predicted from the structure of the first six exons of the cloned P-450IIC2 gene. A small number of fragments in Eco RI and Bam HI digests were observed that hybridized strongly to the full-length probe. Additional bands would be expected for at least the P-450IIC1 gene which is 90% homologous to P-450IIC2 in the protein coding region. Under the stringent hybridization and washing conditions employed, the full-length probe hybridized weakly to one-gene equivalent amounts of P-450IIC1 cDNA fractionated on the same gel. At least two genes closely related to P-450IIC2, in addition to P-450IIC1 are required to account for the number and size of the multiple bands that were observed. Similar results were obtained with P450IIC4 probes for which three strongly hybridizing and several weakly hybridizing bands were observed. P450IIC3 in contrast appeared not to have any other closely related crosshybridizing genes. The results indicate that this subfamily contains about ten members.

The 3′-untranslated region of the P-450IIC2 cDNA is a specific probe for the P-450IIC2 gene since this region of the cDNA is much less conserved among the members of the subfamily than the coding region. The 3′-untranslated probe does not contain Eco RI, Hind III or Pst I sites but does contain a Bam HI site. Thus, a single band for Eco RI, Hind III and Pst I, and two bands for Bam HI should be derived from the P-450IIC2 gene. Southern analysis using the 3′ untranslated region as a probe yielded only the expected single major band for Eco RI and Hind III digested DNA, and two bands for Bam HI digested DNA. These results indicate that no other genes very closely related (>90% homologous) to P-450IIC2 are present in the rabbit genome. This data on the Southern analysis will be published with the gene structures.

4. Isolate and characterize cloned DNA fragments containing the P-450IIC genes. P450IIC2: A rabbit genomic library in Charon 4A was obtained from T.

Mainiatis's laboratory and screened using a mixed probe from P450IIC2 and P450IIC3 cDNA's. From the 40 positive plaques obtained, one hybridized strongly to P450IIC2 cDNA and was characterized in detail. Sequencing of the 17 Kb insert of the cloned DNA established that the DNA corresponded to the first six exons of P450IIC2 and at least 1.5 Kb of 5' flanking region was present. The position of the introns were identical to the position of those previously reported in the rat P450IIB1/2 genes, which are representative of another phenobarbital-inducible subfamily. Family I genes, inducible by 3-methylcholanthrene, have a completely different intron arrangement which suggests that either the 3-MC and PB families are derived from separate ancestral genes or that some selective pressure existed for the divergence of intron positions in the two families. About 800 bp of the 5' flanking region has also been sequenced, This sequence allowed us to determine the sequence of the N-terminal 10 amino acids of P450IIC2, for which the coding sequence was missing in the cDNA clone. The 5' flanking region contains a typical TATA sequence in the promoter region. There were no other obvious homologies between the 5' flanking region of the P450IIC2 gene and P450 genes outside the P450IIC subfamily including the rat P450IIB1/2 genes which are also phenobarbital-inducible and might be expected to have some regulatory sequences in common. The P450IIC2 gene structure has been published (ref. 2).

P450IIC1: A DNA fragment coding for the 5' flanking region and exon 1 of the P450IIC1 gene has also been isolated and the exon and flanking regions sequenced. The mRNA for this gene is not detectable by Northern analysis in control rabbits and increases to levels equivalent to P450IIC2 after phenobarbital treatment and is, therefore, the most responsive gene in the rabbit P450IIC subfamily.

P450IIC3: The complete P450IIC3 gene has been characterized from three different genomic clones. The first clone was obtained from the initial screening described above for P450IIC2. Exonic sequences were sequenced by shotgun cloning the phage DNA into M13 and selecting subclones containing exons by hybridization. This clone contained exons corresponding to exons 7 to 9 (the last three) with positions identical to those in the rat P450IIB1/2 genes. These data were published with the P450IIC2 gene structure. A second lambda phage clone corresponding to the P450IIC3 gene was isolated from the Maniatis library and characterized by DNA sequencing of the exon regions and by restriction mapping to determine distances between the exons. The new clone contained sequences corresponding to exons 2 through 6, however, it did not overlap with the first fragment that had been characterized so that the distance between exon 6 and 7 is not yet known. This gene spans at least 25 kbp. The gene sequence differed from the cDNA sequence in only 2 of 1400 bases and predicted 7 differences of 435 amino acids in the sequence from the protein. We have now obtained from a different library a fragment of DNA containing 8 Kb of 5' flanking region and exons 1 and 2 of P450IIC3. The exon regions and 1 Kb of the 5' flanking region have been sequenced. Southern analysis indicated that there are no other genes very closely related to P450IIC3. This gene is the first constitutive gene of the rabbit subfamily to be characterized.

P450IIC4: In a similar fashion we have characterized a cloned DNA fragment that corresponds to P450IIC4. This fragment of 15 Kbp contains the 5'

flanking region, and the first 5 exons of the gene . There is evidence that there are at least three genes very closely related to P450IIC4, one of which is P450IIC5. Of the 862 exonic bases in the gene sequence there were only 3 differences with the reported P450IIC4 cDNA and 42 with the P450IIC5 sequence providing reasonable assurance that the gene corresponds to P450IIC4. Previous work with the 3′ untranslated region of a P450IIC4 cDNA had indicated that this gene was inducible with phenobarbital. To show that the 5′ P450IIC4 gene sequence was also inducible by phenobarbital, oligonucleotides were synthesized that were specific for either P450IIC5 or P450IIC4. Hybridization of these probes to mRNA from control and phenobarbital-treated livers indicated that the C5 mRNA was not induced and the C4 mRNA was induced by several-fold as expected. We have also mapped the 5′ start site of transcription by protection from single-strand specific nuclease of DNA fragments hybridized to mRNA. A major protected fragment started at an A residue that was about 20 bp upstream from the ATG protein initiator codon and about 25 bp downstream from a consensus TATA sequence. Minor start sites were present 54 bp prior to the first RNA initiation site with a second TATA site present in the appropriate position to direct the initiation of RNA transcription.

P450IIC5: While attempting to obtain P450IIC4 clones, a cloned fragment was obtained that corresponded to P450IIC5 and contained exons 7 to 9.

In addition to the publication of the P450IIC2 gene data (ref. 2), a brief summary of most of this data has been published in a meeting report (ref. 5) and it is expected that two research papers will result, one on the 5′ flanking regions of phenobarbital-inducible forms and one on the complete sequence of P450 IIC3.

5. Evaluate the evolutionary relationships and possible functional significance of structure by comparative analysis of amino acid and nucleotide sequences of the P-450IIC proteins with each other and other P-450's. The identity of the intron locations in the P450IIB and P450IIC subfamilies and the different intron locations in other P450 families have been discussed above. At present it is difficult to explain how the P450 gene families that presumably are derived from a common ancestral gene evolved different intron locations. Most of our other analysis in this area involves the comparison of the 5′ flanking regions of the P450IIC genes in an attempt to identify conserved, possibly regulatory, regions. We have compared the sequences of each pair of P450IIC genes by dot matrix analysis. In the 5′ flanking region there is considerable similarity around the TATA sequence at -20 and three other regions of similarity in the -50 to -150 region between P450IIC4 and either P450IIC1 or C2. Homology out to -400 is apparent when comparing P450IIC1 and P450IIC2. Little homology is noted between P450IIC3 and the other P450IIC genes nor is any homology apparent between the P450IIC genes and those in the P450IIB subfamily. These results indicate that the 5′ flanking region is much less conserved than the coding regions. The homologies retained in the P450IIC1/2/4 genes which are phenobarbital-inducible may have a regulatory role but this remains to be established. Since common regulatory regions should be present in all these genes, at least for liver specific expression, the lack of homology of P450IIC3 with the other genes indicated that the regions of homology for regulation are small and not detectable by this technique. We are continuing analysis of these sequences by searching for known regulatory

regions and will publish this information with the gene sequences.

6. Analyze the regulation of the transcription of the P-450IIC genes introduced into mammalian cells. Preliminary studies on the expression of the P450IIC2 gene after transfection into a rat heptoma cell line, Hepa 1-6, have been done. These studies involved the insertion of the 5′ flanking region of the P450IIC2 gene in front of the gene for the bacterial enzyme, chloramphenicol acetylase transferase (CAT). After transfection of this hybrid into mammalian cells, transcription of the P450IIC2 gene can be assayed by measuring the CAT activity in the transfected cells. As a control, RSVCAT was also transfected into the cells. This construction contains a strong promoter from the Rous sarcoma virus which is expressed in all cells tested so far. The Hepa 1-6 cell line was chosen because P450 levels in the cells had been shown to increase after phenobarbital treatment. Cells transfected with the RSVCAT control had substantial levels of CAT activity indicating that the transfection was successful and DNA had been taken up by these cells. However, no CAT activity was observed in the cells transfected with the P450IIC2-CAT constructions. This result probably indicates that these cells do not express the rat equivalent of P450IIC2.

To attempt to circumvent the apparent inactivity of the P450 promoter in these cells, we have fused a series of fragments from the 5′ flanking regions of P450IIC2 and P450IIC4 to a CAT gene that contained a thymidine kinase (TK) promoter. In principle this approach should allow the identification of regulatory sequences in the P450 genes that modulate the TK promoter. To identify liver specific factors, the expression of these hybrid genes was tested in a hepatoma cell line and a fibroblast cell. The results were uniformly negatively with little or no stimulation of TK promoter activity in either cell line and with no significant differences between the two cell lines. Efforts to develop a viable functional assay for the genes are continuing.

7. Analyze the number and size of nuclear and cytoplasmic RNA's that hybridize to P450IIC cDNA probes. Nothern blotting has been competed on cytoplasmic RNA from all four P450IIC cDNA's. P450IIC3 and P450IIC4 have one major species of mRNA containing approximately 2000 nucleotides. In contrast, P450IIC1 and P450IIC2 contain 4 and 2 species, respectively, ranging in size from 2000 to 4000 nucleotides. The multiple species are thought to result from multiple polyadenylation sites for each mRNA, based in part on the sequence of P450 cDNA clones which contain different polyadenylation sites. This information has been published (refs. 1 & 4).

8. Analyze the kinetics of the induction of P450IIC mRNAs. This analysis has not been done in detail. Measurements of mRNA levels from 6 to 48 hours after phenobarbital treatment suggest that maximal induction occurs by about 24 hours. Similar experiments on transcription of P450IIC1/2 mRNA from 6 to 24 hours after phenobarbital treatment showed that maximal transcription was observed at 6 hours which is consistent with the later peak of mRNA levels.

9. Analyze the tissue distribution and tissue specificity of the induction of P450IIC mRNAs. An analysis by dot blot and Northern blot hybridization was done for all the rabbit P450IIC cDNAs. In liver the mRNA of P450IIC1 was not detectable in control animals and was induced to high levels by phenobar-

bital, the mRNAs for P450IIC2 and P450IIC4 were present in control livers and were induced several-fold by phenobarbital and the mRNA for P450IIC3 was present at the same high levels in untreated and phenobarbital-treated animals. Studies of kidney and lung RNA indicated that only P450IIC2 mRNA was present in the kidney and the level was increased several-fold by phenobartial treatment. These results have been published (ref. 1 & 4).

10. Determine the role of synthesis and degradation in the induction of P450IIC mRNA. To determine whether the changes in mRNA levels could be accounted for by increased transcription of the genes, the rate of transcription was estimated by run-off transcription in isolated nuclei. [α-^{32}P] UTP was used in in vitro nuclear transcription reactions to obtain radiolabeled preinitiated RNA molecules for hybridization to P450IIC cDNA probes immobilized on nitrocellulose filters. The relative rates of transcription of the P-450IIC genes was estimated by the fraction of the total radioactive RNA that bound to the filters. Nuclear transcription reactions were performed simultaneously for liver nuclei of rabbits treated with PB for 0, 6, 12 and 24 hr. The reaction kinetics were monitored by TCA precipitation of aliquots of the transcription reaction. The incorporation of [^{32}P]UMP into total RNA increased rapidly in the first 10 minutes and then leveled off. [^{32}P]UMP incorporation was inhibited in the presence of 1 μg/ml of α-amanitin in the transcription reaction, indicating that it is dependent on RNA polymerase II.

Relative transcription rates of P-450s IIC1, IIC2, IIC3 and IIC4 as a function of PB treatment were determined by isolating total nuclear RNA labeled with [α-^{32}P]UTP and hybridizing it to immobilized recombinant DNA which contains the appropriate P-450 cDNA. The P450 cDNA clones used for the hybridization reactions contained sequences coding for each of the P450s. Filters carrying pTZ18R DNA as a control were incubated under identical conditions and the amount of radioactivity adsorbed to the filter containing control plasmid (pTZ18R) DNA was subtracted from the amount of radioactivity of individual cDNAs. Calculated parts per million were based on the total amount of radioactive RNA incubated with each filter.

To determine reproducibility of the hybridization results, three independent sets of transcriptions were performed. Although some variation was observed, the results of each set indicated that transcriptions of RNAs hybridizing to IIC1, IIC2 and IIC4 probes were maximally induced at 6 hr, and declined thereafter. RNA hybridizing to the IIC3 probe increased slightly in each case without a peak at 6 hr. The specific radioactive RNA bound to the cDNA was averaged for the three individual sets. For P450IIC1/2 an 8- to 9-fold increase 6 hr after PB treatment, that decreased to approximately 4-fold at the 12 and 24 hr time points, was observed. Since the P450IIC1 and P450IIC2 DNAs used in our study are 88% homologous to each other, and the radioactive RNA of both species cross-hybridize under our conditions of hybridization and washing, the induction profiles of nuclear RNA hybridizing to P450IIC1 and P450IIC2 probes are almost identical. In our previous studies, P450IIC1 mRNA was not detected in uninduced rabbit liver, and increased substantially (probably 10- to 20-fold) upon PB treatment. P450IIC2 mRNA was found to be present in control liver and a modest (4-fold) induction was observed after PB administration. The observed 8-fold increase in transcription at 6 hr is consistent with the average increase in mRNA levels of the two genes.

As expected from our previous observation that the levels of P450IIC3 mRNA are not increased by PB treatement, the relative transcription rate of

P450IIC3 gene was only slightly increased after PB treatment.

The steady state level of mRNA which hybridized to the partial P-450IIC4 cDNA, or its 3'-noncoding region, was shown to be present in uninduced liver and increased approximately 4-fold upon PB injection. The transcriptional activity of P450IIC4 increased 5-fold at the 6 hr time point. This activity declined rapidly, and at the 12 hr time point, fell to the uninduced values. The P-450IIC4 cDNA probe is greater than 90% homologous with P450IIC5 cDNA. The measured transcription rate should be an average of at least P450IIC4 and P450IIC5 gene transcription. Since P450IIC5 is known not to respond to phenobarbital administration, the 5-fold induction at 6 hr is somewhat more than expected. This may be explained in part by the transient increase in transcription at 6 hr, resulting in a lower increase in mRNA measured at the 12 and 24 hr time points. Alternatively, other P450 enzymes such as the recently described variant of P450IIC5 (E. Johnson) may cross hybridize with the P450IIC4 probe.

The general conclusion from these transcription studies is that most of the increase in mRNA levels seen after phenobarbital treatment can be accounted by increases in transcription and, thus, by a change in synthesis rather than a change in degradation. These studies will be published with the phenobarbital-inducible gene structures.

D. PUBLICATIONS

1. Leighton, J. K. and Kemper, B. Differential induction and tissue-specific expression of closely realted members of the phenobarbital-inducible rabbit cytochrome P-450 gene family. J. Biol. Chem. 259: 1165-11168 (1984).

2. Govind, S., Bell, P. A. and Kemper, B. (1986) Structure of genes in the cytochrome P-450PBc subfamily: Conservation of intron locations in the phenobarbital-inducible family. DNA 5: 371-382 (1986).

3. Finlayson, M. J., Kemper, B., Browne, N, and Johnson, E. F. Evidence that rabbit cyrochrome P-450 K is encoded by the plasmid pP-450 PBc2. Biochem. Biophys. Res. Commun. 141: 728-733 (1986).

4. Zhao, J., Leighton, J. K. and Kemper, B. Characterization of rabbit cytochrome P450IIC4 cDNA and induction by phenobarbital of related hepatic mRNA levels. Biochem. Biophys. Res. Commun. 146: 224-231 (1987).

5. Kemper, B., Bell, P., Chan, G., Govind, S. and Zhao, J. Structure of rabbit cytochrome P450IIC genes anJ regulation by phenobarbital. In Microsomes and Drug Oxidations, Miners, J. Birkett, D. J., Drew, R. and McManus, M., eds. pp 63-70 Taylor and Francis, London, 1988.

6. Hankinson, O. and Kemper, B. (1987) Meeting report. Cytochrome P450 gene regulation. DNA 6: 515-517 (1987).

The research in the following papers was primarily supported by another grant but used P-450 constructions made in the course of this project and therefore this grant is acknowledged:

7. Mead, D. A., Szczesna-Skorupa, E. and Kemper, B. Single stranded DNA "blue" T7 promoter plasmids: A versatile tandem promoter system for cloning and protein engineering. Prot. Engineering 1: 67-74 (1986).

8. Mead, D. A. and Kemper, B. Chimeric single stranded DNA phage-phagmid cloning vectors. in A Survey of Molecular Cloning Vectors and Their Uses. Rodriguez, R. L. and Denhardt, D. T., eds. pp. 85-102, Butterworth Publishers, Mass, 1988.

In preparation

1. Zhao, J., Chan, G., Govind, S., Bell, P., and Kemper, B. Comparison of the gene structures of phenobarbital-inducible genes in the P450IIC subfamily.

2. Chan, G. and Kemper, B. The structure the rabbit P450IIC3 gene, a constitutive member of the P450IIC subfamily.

E. PERSONNEL

Byron Kemper, Principal Investigator
Shubha Govind, Graduate Research Assistant
Ging Chan, Graduate Research Assistant
Jian Zhao, Graduate Research Assistant
Peter Bell, Visiting Research Specialist
Maria Kyroudis, Visiting Research Specialist

Peter Bell resigned in August 1986 to begin graduate studies at the University of Wisconsin in Cell Biology and will continue working in the P-450 field.

Maria Kyroudis joined the project in 1987.

Degrees awarded:

Shubha Govind, Ph.D., September, 1986
Thesis title: Characterization and transcriptional control of the rabbit cytochrome P-450PBII genes.

Ging Chan, Jian Zhao, anticipated September, 1989.

F. ABSTRACTS/INTERACTIONS

National, International Symposia (invited)

1. Leighton, J. K., DeBrunner-Vossbrinck, B. A., Govind, S. and Kemper, B. Analysis of three putative cytochrome P-450 cDNAs, Sixth International Symposium on Microsomes and Drug Oxidation, Brighton, England. 1984 (poster presented).

2. Govind, S., Leighton, J. K. and Kemper, B. A rabbit cytochrome P-450 phenobarbital gene subfamily, Indian Institute of Science Platimum Jubilee Symposium, International Union of Biochemistry Symposium 134, Bangalore, India. 1984 (paper presented).

3. Kemper, B., Leighton, J., Govind, S., Bell, P. and Zhao, J. Genes of a phenobarbital-inducible P-450 subfamily: structural analysis, differential inducibility and tissue-specific expression, P-450 Gene Workshop (N.I.H.), Airlie, VA. 1985 (paper presented).

4. Govind, S., Bell, P. A. and Kemper, B. Structure of a phenobarbital-inducible cytochrome P-450 gene, Fourth Summer Symposium in Molecular Biology, University Park, PA 1985 (poster presented).

5. Kemper, B., Leighton, J. K., Govind, S., Bell, P., Chan, G. and Zhao, J. Structure and expression of a subfamily of the phenobarbital-inducible cytochrome P-450 gene family, Fourth International Symposium on Comparative Biochemistry, Janssen Research Foundation, Beerse, Belgium, 1985 (paper presented).

6. Finlayson, M. J., Kemper, B., Browne, N. and Johnson, E. F. (1986) Evidence that rabbit cytochrome P-450 K is encoded by the plasmid pPBc2. Fed. Proc. 45: 1854 (poster presented).

7. Govind, S., Bell, P. A., Zhao, J., Chan, G. and Kemper, B. (1986) Structure of genes in the cytochrome P-450PBc subfamily. Fed. Proc. 45: 1855 (poster presented).

8. Kemper, B., Govind, S., Bell, P. A., Zhao, J., Chan, G. and Szczesna-Skorupa, E. Regulation of gene expression and cellular locatization of the rabbit members of the cytochrome P450IIC subfamily. The Second International Workshop on P450 Gene Regulation at Airlie, Virginia sponsored by the NIH, April, 1987 (Paper presented).

9. Kemper, B., Bell, P., Chan, G., Govind, S. and Zhao, J. Structure of rabbit cytochrome P450IIC genes and regulation by phenobarbital. The Seventh International Symposium on Microsomes and Drug Oxidations at Adelaide, Australia, August, 1987 (Paper presented).

Seminar invitations:

1. Department of Molecular and Cellular Biology, University of Oregon, April 1987.

2. Department of Physiology and Biophysics, Case Western REserve, February, 1988.

G. INVENTIONS

None